

Surgery and injection of viral vector

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 An abbreviated version of this protocol was published in eLIFE in Jul 2018

Focal optogenetic suppression in macaque area MT biases direction discrimination and decision confidence, but only transiently

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Detailed protocol







Materials:

quartz glass micropipette w preattached connector (Thomas Recording GmbH)
tungsten microelectrode, epoxy coated (FHC, Inc.)
23 ga. thin-wall stainless steel hypo tubing, cut + sharpened (transdural guide tube)
hydraulic or motorized microdrive
10 or 25 ul Hamilton syringe (700 Series or GasTight) w 27 ga flat-tip needle
syringe pump
medical grade tubing (e.g. Tygon Microbore, 0.01-0.015" I.D.)
1cc syringe & short 27 ga needle for loading the tubing
silicone fluid (<http://www.clearcoproducts.com/pdf/volatile/NP-PSF-1cSt.pdf>) or other low viscosity oil
UV tracer dye (e.g. <https://www.amazon.com/gp/product/B005P3FHQO/>)
UV ('black light') flashlight
virus aliquots, stored at -80C until ready to use

Steps:

Insert & align electrode and micropipette within guide tube.
Glue electrode and pipette together at multiple points with a tiny amount of cyanoacrylate, applied under dissecting scope using a fine tool.
Attach guide tube and back of 'injectrode' to microdrive in standard fashion. If desired, outer surfaces of injectrode can be high-level disinfected at this point (e.g. Cidex, Metricide).
Using 1cc syringe and needle, load a short stretch of the Tygon tubing (sufficient to bridge the distance from syringe pump to monkey) with UV-dyed silicone fluid.
Load Hamilton syringe as well, then connect Hamilton syringe to other end of tubing, removing all bubbles (see photo 1).
Disconnect 1cc syringe and attach tubing to micropipette connector (photo 2).
Thaw virus on ice. Draw ~10 ul into a standard pipette tip, then eject and trim the wide end of the tip as needed (photo 3).
Mount microdrive assembly above pipette tip containing virus, then advance injectrode tip into thawed virus solution.
Use syringe pump to slowly draw up virus into injectrode and tubing (photo 4), adjusting position of injectrode tip to avoid introducing bubbles.
Retract injectrode into guide tube and proceed with insertion into the brain using standard methods. Some positive pressure (slowest possible syringe pump setting) may be advisable to prevent blockage while advancing.
Perform injections as described in the manuscript, using the UV flashlight to confirm movement of the fluid/virus interface in the tubing.

Related files

 Fetsch_photo1.jpg	
 Fetsch_photo2.jpg	
 Fetsch_photo3.jpg	
 Fetsch_photo4.jpg	

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fetsch, C. R. and Shadlen, M. (2022). Surgery and injection of viral vector. Bio-protocol Preprint. [bio-protocol.org/prep1515](https://doi.org/10.21203/rs.3.rs-1515).
2. Fetsch, C. R., Odean, N. N., Jeurissen, D., El-Shamayleh, Y., Horwitz, G. D. and Shadlen, M. N.(2018). Focal optogenetic suppression in macaque area MT biases direction discrimination and decision confidence, but only transiently. eLIFE. DOI: [10.7554/eLife.36523](https://doi.org/10.7554/eLife.36523)

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